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Amolops putaoensis Gan, Qin, Lwin, Li, Quan, Liu & Yu, 2020, a newly recorded torrent frog for China

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Abstract

Amolops putaoensis is a recently described torrent frog species from *A. monticola* group that is known only from its type locality, northern Myanmar. We compared morphology and mitochondrial DNA sequence data from ten recently collected adult male specimens from the upper Dulong River System in Gongshan County, Yunnan Province, China, to the original description of *A. putaoensis*. Both datasets strongly supported referring the Chinese specimens to *A. putaoensis*, extending the known range of this species by approximately 133.7 km distance into China. Molecular phylogenetic analyses recovered *A. putaoensis* to be closely related to *A. aniqiaoensis*, *A. kohimaensis*, *A. monticola*, and *A. adicola*. We use the newly collected Chinese specimens to expand the morphological description of the species.

Key Words

Dulong River System, herpetofauna, national new record, Ranidae

Introduction

The torrent or cascade frogs of the genus *Amolops* Cope, 1865 inhabit swift, rocky streams in mountainous regions of Asia, across Indo-Burma, including northeastern India, Southeast Asia, and southern continental China. Species groups in this genus were revised to ten (*A. chayuensis* group, *A. daiyunensis* group, *A. hainanensis* group, *A. larutensis* group, *A. mantzorum* group, *A. marmoratus* group, *A. monticola* group, *A. ricketti* group, *A. spinapectoralis* group, *A. viridimaculatus* group) by Jiang et al. (2021), among these, the *A. monticola* group currently contains 22 species within a monophyly. As a newly discovered *monticola* complex species, *A. putaoensis* Gan, Qin, Lwin, Li, Quan, Liu & Yu (2020) has been indicated to occur only around its type locality in northern Myanmar.

Our fieldwork in early July 2019 in the upper Dulong River system in Gongshan County, Yunnan Province, China (27.669949°N, 98.268219°E, 1281 m elev.), near to its border with Tibet Province, revealed ten adult males of *Amolops* near a shallow sandy-bottom stream with rocky banks (Fig. 1). All specimens were humanely handled and euthanized, then preserved in

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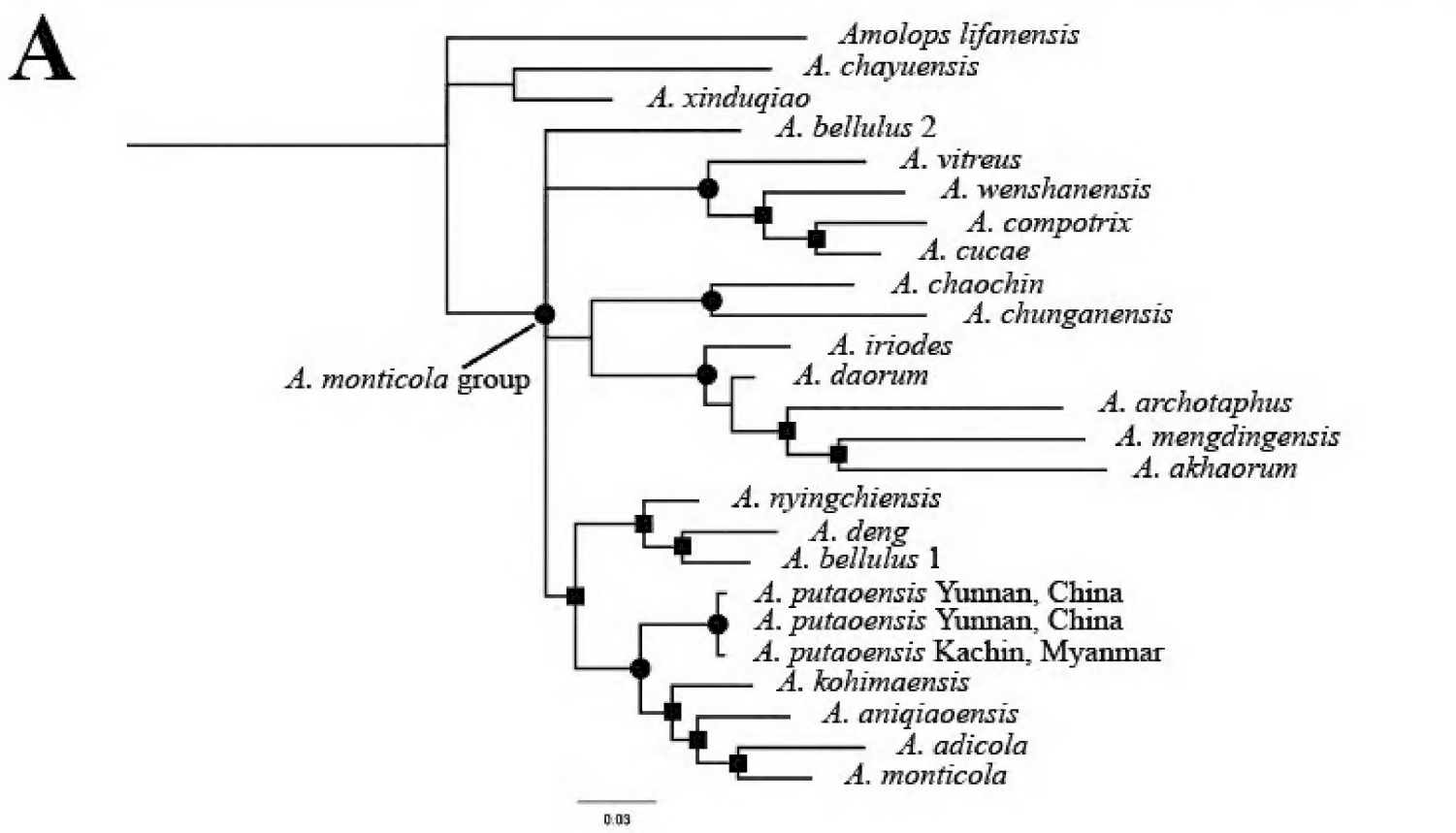

Figure 1. Known localities and molecular phylogenetic relationships of *Amolops putaoensis*. **A.** The type locality (yellow star) at Putao County, Kachin State, Myanmar and the new locality (red star) at Dulongjiang Village, Gongshan County, Yunnan Province, China. Map generated by ArcGIS Pro; **B.** Phylogenetic tree based on Bayesian inference of a fragment of the mitochondrial COI gene. Branches support values of posterior probability (PP) and bootstrap supports (BS) were performed, where the strong supports found in represented Circles represent very strong nodal support of posterior probabilities (PP) of 1.0 and bootstrap support (BS) from a separate maximum likelihood analysis of 100, and squares represent strong nodal support of PP ≤ 0.95 and BS ≤ 70, with lower support values not shown.

75% ethanol, with liver tissues transferred into 95% ethanol and stored at -20°C for molecular analysis. Voucher specimens were deposited in the Kunming Institute of Zoology (KIZ), Yunnan, China. In this study, taxonomy of these specimens was investigated based on morphological comparison and molecular phylogenetic analyses. Both morphological and molecular datasets corroborate referring these specimens to the recently described *A. putaoensis* that was known only from northern Myanmar, extending the known range of *A. putaoensis* by a distance of approximately 133.7 km, and representing a new addition to the known anuran fauna of China.

Methods

Morphological terminology follows Fei et al. (2009). All 10 adult male specimens were measured (Suppl. material 1) using slide calipers to the nearest 0.1 mm: snout-vent length (**SVL**); head length from the tip of snout to rear of the jaws (**HDL**); maximum head width at commissure corner of jaws (**HDW**); internarial distance (**IND**); snout-length from the tip of snout to the anterior corner of the eye (**SNT**); horizontal diameter of exposed portion of the eyeball (**EYE**); interorbital distance at the narrowest point (**IOD**); horizontal diameter of tympanum (**TMP**); tympanum-eye distance from the anterior edge of tympa-

num to posterior corner of the eye (**TEY**); femur length from the vent to outer edge of knee (**FEM**); hand length from the base of palm to the tip of finger III (**HND**); maximum width of finger III digital disc (**F3DSC**); foot length (**FTL**); manus length from the tip of third digit to proximal edge of inner palmar tubercle (**ML**); pes length from the tip of toe IV to proximal edge of inner metatarsal tubercle (**PL**); maximum width of upper eyelid (**UEW**); forearm and hand length (**FAHL**); maximum width of forearm (**FAW**); length of tarsus and foot (**TFL**); width of finger II digital disc (**F2D**); and width of toe IV digital disc (**T4D**).

Genomic DNA was extracted from liver tissues of two individuals (KIZ 035035, KIZ 035037) using the KaTaRa DNA purification kit. A fragment of the mitochondrial cytochrome C oxidase subunit 1 gene (COI, Che et al. 2012) was amplified by the polymerase chain reaction (PCR). Amplified PCR products were sequenced using an ABI 3730 automated sequencer. Newly-generated sequences were edited to consistent length using MEGA X (Kumar et al. 2018) and deposited in GenBank under accession numbers OP831318 and OP831319 (vouchered specimens see Suppl. material 2).

Homologous sequences of 21 species in the *A. monticola* group were obtained from GenBank, including the holotype of *A. putaoensis*. Homologous sequences of *A. chayuensis* (*A. chayuensis* group), *A. xinduqiao* and *A. lifanensis* (both in the *A. mantzorum* group) were also downloaded from GenBank for use as outgroups (Suppl. material 2). The newly-generated and downloaded sequences were aligned by MEGA X. Mean pairwise uncorrected genetic distances (*p*-distances) between the Gongshan specimens and other closely-related *Amolops* species were calculated by MEGA X. Phylogenetic trees were reconstructed using Bayesian inference (BI) by MrBayes 3.2.7a. and the Maximum Likelihood (ML) criterion performed by RAxML NG v0.9.0. (Kozlov et al. 2019). The GTR+I+G model was selected as the best substitution model for each codon position by JModelTest 2 (Guindon and Gascuel 2003; Darriba et al. 2012). The BI analyses used Metropolis Coupled Markov Chain Monte Carlo (MCMC) with three heated chains and one cold chain for 5,000,000 generations and sampled every 1,000 generations, with every 1,500 generations with the first 25% of samples discarded as burn-in. The potential scale reduction factor (PSRF > 1) and the average standard deviation of split frequencies (ASDSF < 0.01) were used to evaluate topological and branch-length convergences, respectively. Confidence in tree nodes was assessed by posterior probabilities (PP) (Huelsenbeck and Ronquist 2001). ML analyses were performed with nodal support based on 1,500 bootstrap (BS) replicates. Relationships with PP ≥ 0.95 and BS ≥ 70 were considered to be strongly supported.

Results

Molecular analyses

The aligned COI dataset contained a total of 570 nucleotide base pairs (bp), with 214 variable positions and 168 parsimony informative sites. The uncorrected pairwise distance (*p*-distance) between the Gongshan specimens and the holotype of *A. putaoensis* was only 0.005 (Suppl. material 3). The BI and ML analyses obtained consistent topologies and most nodes were supported well. Both analyses placed the Gongshan specimens and the holotype of *A. putaoensis* in a single clade, and it was recovered with strong support as sister to the clade containing *A. aniqiaoensis*, *A. kohimaensis*, *A. adicola*, and *A. monticola* (Fig. 1).

Morphological analyses

The Gongshan specimens closely matched the original description of *A. putaoensis* by Gan et al. (2020) (Suppl. materials 2, 4), with two notable exceptions. First, the Gongshan specimens have larger body sizes, with male SVL 47.5–51.3 mm, vs. 37.6–40.2 mm in the type series. Second, the Gongshan specimens have a pair of external subgular vocal sacs (Fig. 2G), stated to be internal vocal sacs in the type series by Gan et al. (2020). As this character is not visible in the figures in the original description, we assume that the type series was not calling when collected and this character was therefore overlooked by Gan et al. (2020). The description of *A. putaoensis* is expanded using the Gongshan specimens, as follows.

Taxonomic account

Family Ranidae

Amolops putaoensis Gan, Qin, Lwin, Li, Quan, Liu & Yu, 2020

Fig. 2

Specimens examined (n = 10 adult males). KIZ 035035–035044 (Fig. 2), collected from Dulongjiang Village, Gongshan County, Yunnan Province, China (27.66995°N, 98.26822°E, 1218 m elev.) by Yu-Fan Wang, Xiao-Long Liu and Zhi-Yong Yuan on 4 July 2019.

Expanded diagnosis. A member of the *A. monticola* group having males (n = 13) with side of head dark with light-colored upper lip stripe extending to axilla; distinct dorsolateral fold; weak to moderately developed supratympanic fold; no outer metatarsal tubercle; circummarginal groove on tip of first finger; SVL 37.6–51.3 mm; head length slightly short or subequal to head width, with HDL : HDW 0.87–1.02; smooth dorsal skin, with dense, tiny dermal granules distributed from posterior margin of nostril to cloaca; tympanum diameter less than half of eye diameter; distinct transverse bands on dorsal surfaces of limbs; venter greyish white, with irregular spots from throat to mid-belly; visible pineal body; vomerine teeth; nuptial pads; external subgular vocal sacs; and maxillary gland.

Expanded description. In life, dorsum with smooth skin; dorsum colored in brown with black irregular spots from snout to vent; lateral region lemon yellow to olive green, dorsal and lateral coloration divided by a distinct dorsolat-

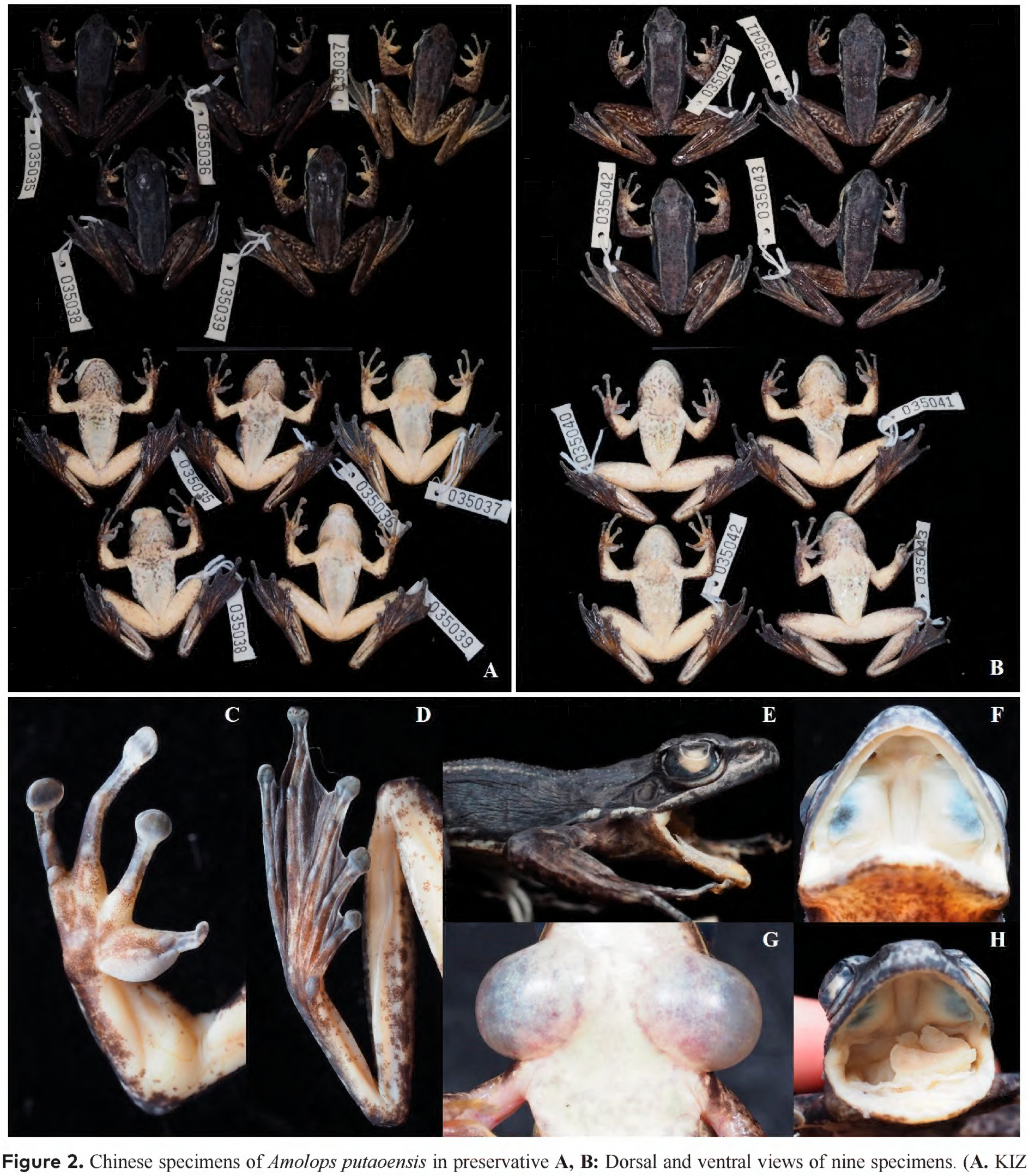

Figure 2. Chinese specimens of *Amolops putaoensis* in preservative **A, B:** Dorsal and ventral views of nine specimens. (**A.** KIZ 035035–035039; **B.** KIZ 035040–035043); **C–H:** Ventral hand, ventral foot with webbing, lateral side of head, a pair of external subgular vocal sacs, a pair of short oblique vomerine teeth, and heart-shape notched tongue. Photo credit: Yin-Peng Zhang.

eral gland extending from posterior margin of eye to near vent; tiny dermal granules extending from posterior margin of nostrils and eyelid to near vent, absent from lateral and ventral regions of body; tarsal and skin folds absent; venter greyish white, with irregular spots from throat to chest. In preservative (75% ethanol), dorsal coloration faded to dark bluish-green and ventral coloration to creamy yellow.

Expanded variation. In life, dorsal coloration varied from yellowish brown to dark brown; lateral coloration varied from lemon yellow to olive green; irregular spots on flank rather distinct or weak; number of transverse bands on forearm ranged from 4–8; and number of transverse bands on tibia ranged from 6–7 (Fig. 3).

Expanded comparisons. Gan et al. (2020) did not compare *A. putaoensis* with the closely related *A. kohimaensis* (Fig. 1) and only distinguished *A. putaoensis* from *A. aniqiaoensis* on the basis of male body size, a character that does not hold up with the addition of the Gongshan specimens. Its other two relatives, *A. monticola* and *A. adicola* (Fig. 1), were poorly known or undescribed, respectively, at the time of the description of *A. putaoensis*. With the addition of the Gongshan specimens, *A. putaoensis*

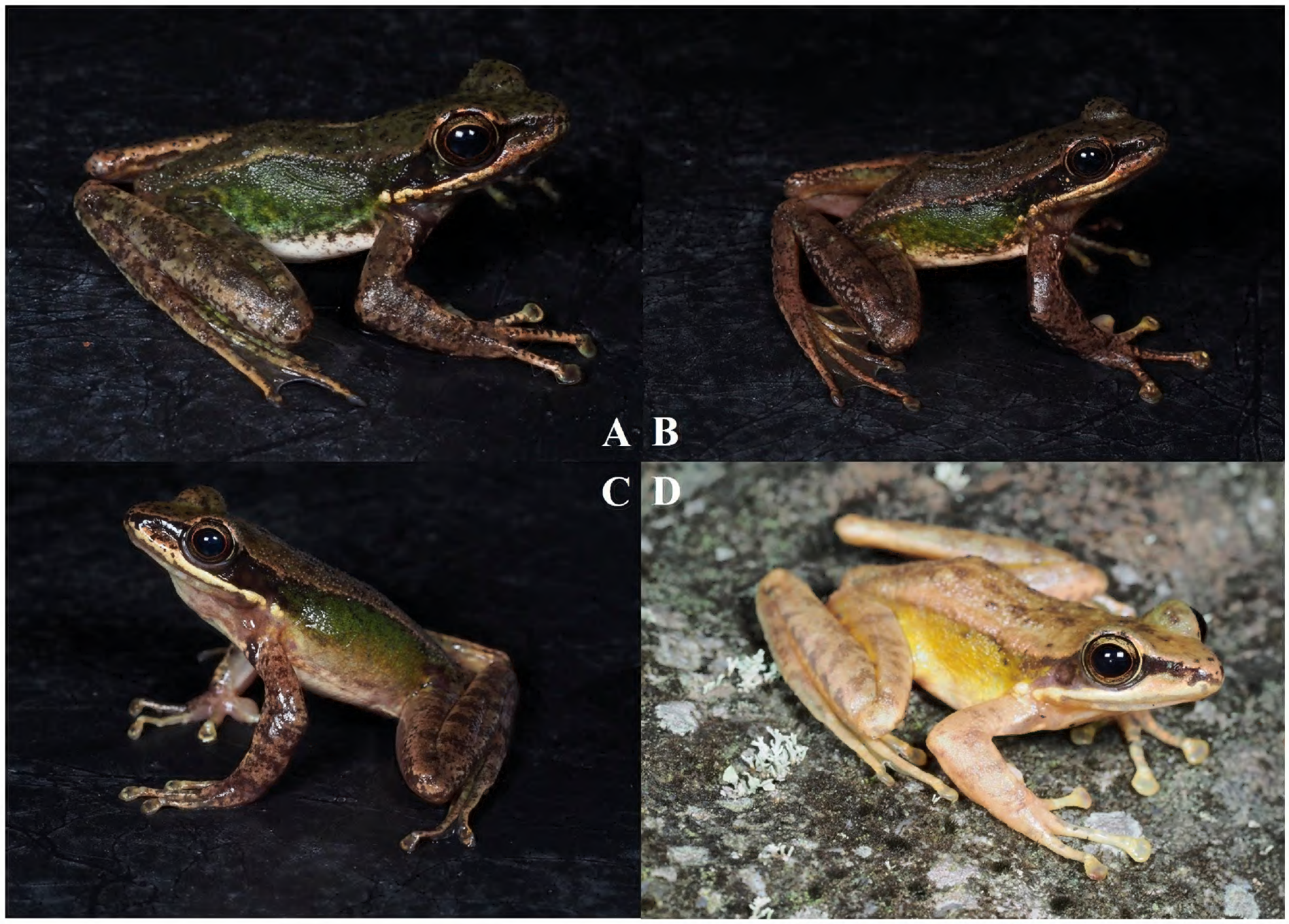

Figure 3. Chinese specimens of *Amolops putaoensis* in life (**A.** KIZ 035044; **B.** KIZ 035043; **C.** KIZ 035042; **D.** KIZ 035046). Photo credit: Xiao-Long Liu.

can be distinguished from *A. kohimaensis* by lacking an outer metatarsal tubercle (vs. present); and having distinct dorsolateral folds (vs. absent). *Amolops putaoensis* can be distinguished from *A. aniqiaoensis* by having distinct transverse bands on dorsal surfaces of limbs (vs. absent or indistinct patterns); and lacking a "/ \"-shaped mark on chest (vs. present). *Amolops putaoensis* can be distinguished from *A. monticola* by lacking an outer metatarsal tubercle (vs. present); and having circummarginal grooves on finger I (vs. absent). *Amolops putaoensis* can be distinguished from *A. adicola* by having distinct transverse bands on dorsal surfaces of limbs (vs. reticulation); and having ventral spots from throat to mid-belly (vs. absent).

Expanded distribution. *Amolops putaoensis* is known only from its type locality in Putao County, Kachin State, northern Myanmar, and from our newly recorded locality in Dulongjiang Village, Gongshan County, Yunnan Province, China, approximately 133.7 km northeast of the type locality.

Discussion

Our morphological and mitochondrial DNA data strongly support referring the newly collected specimens at Gongshan County, Yunnan Province, China as the new national record of *Amolops putaoensis*. Our study extends the geographic range of the species northeast into China and demonstrates that *A. putaoensis* is closely related to *A. kohimaensis* from northeastern India (Biju et al. 2010), *A. aniqiaoensis* from Medog, Tibet, China (Che et al. 2020), and *A. monticola* and *A. adicola* from northeastern India (Patel et al. 2021). Unfortunately, both the original description (Gan et al. 2020) and our study did not find females or larvae of *A. putaoensis*, or document advertisement calls of males. Hence, additional research into the natural history of *A. putaoensis* is warranted.

Acknowledgements

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Reference

Biju SD, Mahony S, Kamei RG (2010) Description of two new species of torrent frog, *Amolops* Cope (Anura: Ranidae) from a degrading forest in the northeast Indian state of Nagaland. Zootaxa 2408: 31–46. https://doi.org/10.11646/zootaxa.2408.1.2

Che J, Chen HM, Yang JX, Jin JQ, Jiang K, Yuan ZY, Murphy RW, Zhang YP (2012) Universal COI primers for DNA barcoding amphibians. Molecular Ecology Resources 12: 274–258. https://doi.org/10.1111/j.1755-0998.2011.03090.x

Che J, Jiang K, Yan F, Zhang YP (2020) Amphibians and Reptiles in Tibet. Beijing: Science Press, 176–223. [In Chinese]

Cope ED (1865) Sketch of the primary groups of Batrachia s. Salientia. Natural History Review 5: 97–120.

Gan YL, Qin T, Lwin Y, Li GG, Quan RC, Liu S, Yu GH (2020) A new species of *Amolops* (Anura: Ranidae) from northern Myanmar. Zoological Research 41(6): 734–740. https://doi.org/10.24272/j.issn.2095-8137.2020.125

Guindon S, Gasuel O (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Systematic Biology 52: 696–704. https://doi.org/10.1080/10635150390235520

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinfomatics 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Jiang K, Ren J, Lyu ZT, Wang D, Wang Z, Lv K, Wu JW, Li JT (2021) Taxonomic revision of *Amolops chunganensis* (Pope, 1929) (Amphibia: Anura) and description of a new species from southwestern China, with discussion on *Amolops monticola* group and assignment of species groups of the genus *Amolops*. Zoological Research 42(5): 574–591. https://doi.org/10.24272/j.issn.2095-8137.2021.107

Kozlov A, Darriba D, Flouri T, Morel B, Stamatakis A (2019) RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics 35: 4453–4455. https://doi.org/10.1093/bioinformatics/btz305

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology of Evolution 35: 1547–1549. https://doi.org/10.1093/molbev/msy096

Patel NG, Garg S, Das A, Stuart B, Biju SD (2021) Phylogenetic position of the poorly known Montane Cascade Frog *Amolops monticola* (Ranidae) and description of a new closely related species from Northeast India. Journal of Natural History 55(21–22): 1403–1440. https://doi.org/10.1080/00222933.2021.1946185

Supplementary material 1

Morphological measurements of *Amolops putaoensis* from a newly collected locality in Yunnan, China and its type locality in Kachin, Myanmar

Authors: Yin-Peng Zhang, Xiao-Long Liu, Bryan L. Stuart, Dong-Yi Wu, Yu-Fan Wang, Jing Che, Zhi-Yong Yuan

Data type: Word file

Explanation note: Data for the type series taken from Gan et al. (2020). A "/" indicate data not available from typed series.

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Supplementary material 2

Mitochondrial COI gene sequences of *Amolops* species used in this study

Authors: Yin-Peng Zhang, Xiao-Long Liu, Bryan L. Stuart, Dong-Yi Wu, Yu-Fan Wang, Jing Che, Zhi-Yong Yuan

Data type: Word file

Explanation note: Obtained GenBank information.

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Supplementary material 3

Uncorrected pairwise distances (p-distances) in the mitochondrial COI gene among selected members of the *Amolops monticola* group compared with *A. putaoensis*

Authors: Yin-Peng Zhang, Xiao-Long Liu, Bryan L. Stuart, Dong-Yi Wu, Yu-Fan Wang, Jing Che, Zhi-Yong Yuan

Data type: Word file

Explanation note: Comparisons of *A. putaoensis* at a newly collected locality in Yunnan, China and its type locality in Kachin, Myanmar are indicated in bold.

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Supplementary material 4

Morphological comparisons of *Amolops putaoensis* from a newly collected locality in Yunnan, China and its type locality in Kachin, Myanmar

Authors: Yin-Peng Zhang, Xiao-Long Liu, Bryan L. Stuart, Dong-Yi Wu, Yu-Fan Wang, Jing Che, Zhi-Yong Yuan

Data type: Word file

Explanation note: Data for the type series taken from Gan et al. (2020).

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